

THE MECHANISM OF INHERITANCE IN OENOTHERA

BY STERLING EMERSON

PART I.—MULTIPLE-FACTOR INHERITANCE IN
CROSSES BETWEEN *OENOTHERA FRANCISCANA*
AND *OENOTHERA GRANDIFLORA*.

PART II.—THE INHERITANCE OF CERTAIN
CHARACTERS IN OENOTHERA CROSSES OF
DIFFERENT CHROMOSOME CONFIGURATIONS

A Dissertation Submitted in Partial Fullfillment of the Requirements
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ANN ARBOR

CHROMOSOME CONFIGURATION IN
A DWARF SEGREGATE FROM
*OENOTHERA "FRANCISCANA
SULFUREA"*

STERLING H. EMERSON

MULTIPLE-FACTOR INHERITANCE
IN CROSSES BETWEEN *OENOTHERA
GRANDIFLORA* AND *OENOTHERA
FRANCISCANA*

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CHROMOSOME CONFIGURATION IN A DWARF SEGREGATE FROM *OENOTHERA* "*FRANCISCANA* *SULFUREA*"*

STERLING H. EMERSON

CONTENTS

Introduction	
Material and methods	
Description of chromosomes	
Discussion	
Literature cited	
Explanation of plate	
Plate	

INTRODUCTION

THE *Oenothera* known as hybrid "*franciscana sulfurea*" arose in 1914 as a single light-flowered plant in the second generation of the cross *Oe. biennis* $\times$ *Oe. franciscana*, which had been made by Professor Davis in 1912 (Cleland, 1924). Aside from the difference in flower color (which in the hybrid is identical with the sulphur flower color of *Oe. biennis sulfurea*), hybrid "*franciscana sulfurea*" resembles *Oe. franciscana* in most characteristics. It differs in having the green buds of *Oe. biennis*, in its heterozygous character, and in chromosome configuration.

Cleland (1923, 1924) has described the configuration of chromosomes at meiosis in this hybrid, finding, in the late prophase, a ring of twelve chromosomes and a single pair. At metaphase, the paired chromosomes separated normally, while the chromosomes constituting the ring separated in the characteristic zigzag manner typical of *oenotheras* with non-pairing chromosomes. I have since observed the same arrangement and separation of chromosomes in later generations of the same material.

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When inbred, hybrid "*franciscana sulfurea*" regularly gives segregating progenies, consisting chiefly of the parental form with about one third of the plants of a new dwarf type. The dwarf segregate differs from the parent not only in stature but in bud color (being red-budded as is *Oe. franciscana*), and in being apparently homozygous for all characters.

MATERIAL AND METHODS

The plants used in this investigation were inbred descendants from a dwarf plant isolated from a culture of hybrid "*franciscana sulfurea*" by Professor B. M. Davis.

Anthers were fixed for fifteen minutes in a solution consisting of 1 gram picric acid and 5 ml. dichloroacetic acid in 100 ml. of absolute alcohol. A volume of absolute alcohol equal to the volume of killing solution used was then added and after fifteen minutes the solution was decanted off and the anthers placed in absolute alcohol. A mixture of one half xylol and one half cedar oil was then added in a layer under the alcohol and the buds were allowed to settle into this mixture. The anthers were then placed in fresh xylol-cedar oil mixture, and melted paraffin was added. The anthers were imbedded directly in hard paraffin with only two changes of paraffin. The entire process of fixing and imbedding required considerably less than 24 hours.

Sections were cut at 15 microns to insure entire nuclei and stained by the iodine-crystal violet method described by Clausen (1926).

In some pollen sacs the microsporocytes were badly shrunken, but, for the most part, very good preparations were obtained by this short method.

DESCRIPTION OF CHROMOSOMES

In the late prophase, the chromosomes appear as seven pairs, the members of any pair usually forming a small ring (Pl. XXXIV, Figs. 1-5). Some of the chromosomes often appear to be clumped, in which cases the seven pairs cannot be seen distinctly. In those nuclei in which all fourteen chromosomes can be recognized with certainty, they appear as seven pairs completely unassociated.

Metaphase is extremely regular in all respects. Ordinarily

the chromosomes lie too close together for recognition of all seven pairs (Pl. XXXIV, Fig. 6). It is only when the plane of the section is slightly oblique to the spindle that the seven pairs may be seen distinctly (Pl. XXXIV, Figs. 7-8).

In some prophase nuclei, a small deeply staining body was seen (Pl. XXXIV, Figs. 2, 4, 5). This body was completely absent in some nuclei and was never seen at metaphase. It resembled a chromosome fragment, but was more probably formed by the degeneration of the nucleolus.

DISCUSSION

The case described in this paper makes the third in which a heterozygous form of *Oenothera* is known to have many non-pairing chromosomes and to give rise to a homozygous form with chromosomes all normally pairing.

Cleland (1925) has described the cytological situation in the case of mut. *rubrinervis* and its segregate mut. *deserens*. Mut. *rubrinervis* was found to have a circle of six chromosomes and four pairs, while mut. *deserens* had seven pairs of chromosomes. The genetics of these forms is briefly as follows: Mut. *rubrinervis* is heterozygous; when self-pollinated it produces two types of offspring, the parent type and mut. *deserens* (De Vries, 1916). Mut. *rubrinervis* is also heterogametic as shown by the twin hybrids in crosses with *Oe. muricata* (*Auct. non L.*) (De Vries, 1913), and with *Oe. biennis* (Renner, 1917, 1918, 1919). Mut. *deserens* seems to be completely homozygous and produces but one type of gamete.

The situation for mut. *rubricalyx* and its segregate mut. *latifrons* is much the same. Mut. *rubricalyx* has been found by Cleland (1925) to have a circle of eight chromosomes and three chromosome pairs. Mut. *latifrons* has all pairing chromosomes.

The case described in this paper is in some respects more striking than the two just mentioned. The heterozygous form, hybrid "*franciscana sulfurea*," has but one pair of chromosomes. It is known to produce two types of gametes, only one of which functions as pollen. It is also known to be heterozygous for three independent characters. The dwarf segregate is known to produce but one type of gamete and is completely homozygous for

all characters that can be tested. The experiments on which these statements are based will be published shortly.

The relation shown to exist between the chromosome configuration and the relatively homozygous or heterozygous condition in the forms contrasted above helps to substantiate the general contention that the genetic behavior of the *Oenotheras* is in some way dependent on the chromosome arrangement at the reduction division.

UNIVERSITY OF MICHIGAN

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EXPLANATION OF PLATE XXXIV

All figures were drawn with the aid of a camera lucida. A Spencer compound binocular microscope and a Zeiss 90-1.4 apochromatic objective and 20× compensating ocular were used.

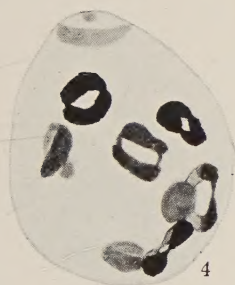
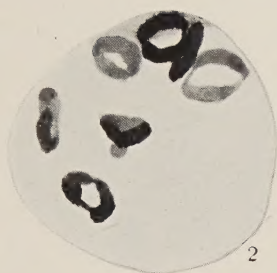
FIG. 1. Prophase, after second contraction, showing seven pairs of chromosomes.

FIGS. 2-5. Slightly later than Figure 1. All show seven pairs of chromosomes.

FIG. 6. Typical metaphase showing regular pairing of chromosomes. Not all pairs can be definitely recognized.

FIGS. 7 and 8. Metaphase. The sections are cut somewhat oblique to the spindle, allowing all seven pairs to be seen.

PLATE XXXIV





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MULTIPLE-FACTOR INHERITANCE IN
CROSSES BETWEEN *OENOTHERA*
GRANDIFLORA AND *OENOTHERA*
FRANCISCANA *

STERLING H. EMERSON

CONTENTS

Introduction	
Material	
Scope of experiments	
Genetic analysis	
Time of flowering	
Growth habit	
Bud color	
Sepal tip length	
Stem tip color	
Coloring of stems at base of plant	
Length of floral bracts	
Length of buds	
Pubescence (thick-walled)	
Pubescence (thin-walled)	
Evidence of correlation	
Correlation between five characters	
Characters independently inherited	
Discussion	
Multiple-factor interpretation	
Relation of multiple factors to the paired condition of chromosomes	
Literature cited	
Explanation of plates	
Plates	

INTRODUCTION

Material

THE original crosses between *Oenothera franciscana* and *Oenothera grandiflora* were made by Professor Davis in the summer of 1921 and the first generations were grown at Ann Arbor in the summer of 1923, at which time Professor Davis kindly turned over

* Paper from the Department of Botany of the University of Michigan, No. 287.

to me all the material of these crosses, including seed from pollinations made by him that summer. Succeeding generations were grown at Ann Arbor in 1924 and 1925.

The strain of *Oe. grandiflora* used in these crosses was one that had been grown by Professor Davis since 1909 in an inbred line, coming originally from seed collected for him at Dixie Landing, near Tensaw, Alabama (Davis, 1911). This is the strain of *Oe. grandiflora* that was used in the early cytological studies (Davis, 1909).

The plants of *Oe. franciscana* used were the descendants in an inbred line from the original collection by Dr. Charles Piper Smith (Davis, 1916; Bartlett, 1914). This strain has also been analyzed cytologically (Cleland, 1922).

Scope of Experiments

At the time these experiments were planned, it was hoped that crosses between these two species would prove more simple in their genetic behavior than most *oenotheras* because of the large number of normally pairing chromosomes in both parents. The studies of Davis (1919) showed that *Oe. grandiflora* had seven pairs of chromosomes. Cleland has reexamined this species with the same result. *Oe. franciscana* has been found by Cleland (1922) to have five pairs and a ring of four chromosomes. Apparently the plants of the first generations of crosses between these two species may have the chromosome configuration of either parent. Only one plant (the parent of culture 30) was examined by me; this had seven pairs of chromosomes. Cleland has examined several plants of the first generations of reciprocal crosses and found a ring of four and five pairs of chromosomes in each.

The genetic behavior to be reviewed in this paper shows certain peculiarities doubtless due to the paired condition of the chromosomes. The material did not prove well adapted to genetic analysis, however, because of the absence of sharply segregating characters. For this reason the experiments were not pushed to a definite conclusion, but since the behavior noted is unusual for the *oenotheras*, it seems well to publish the findings of these crosses in their incomplete form.

I wish to take this opportunity to thank Professor Davis for the original material and for his kind permission to utilize his notes on the lines before they were taken over by me.

GENETIC ANALYSIS

The two species used in the crosses described in this paper differ from each other in a great many characteristics, the inheritance of several of which was studied. No attempt will be made to give a detailed description of either of the parental forms or of the hybrid generations, but each will be contrasted separately for each characteristic studied.

Not all plants in the second generation cultures were classified for each character studied. The number of individuals listed for a given culture indicates only the number that have been classified for the particular character under discussion.

Time of Flowering

Oe. franciscana is ordinarily one of the first species in our collection to flower, while *Oe. grandiflora* is about the latest. Under our ordinary methods of culture, sowing the seeds in the greenhouse in January or February and transplanting to the field in May, *Oe. franciscana* will begin to flower as early as the first week in July. *Oe. grandiflora* under similar conditions rarely flowers before the last week in August, at which time *Oe. franciscana* is nearly past its flowering season. Most plants of *Oe. grandiflora* fail to ripen seeds before frost at Ann Arbor.

The first generation plants were intermediate between the two parents for time of flowering. The reciprocal F_1 cultures were uniform in this respect, all coming into flower about the end of July. The only exceptions were the weaker chlorotic plants of the cross *franciscana* $\times$ *grandiflora*, which were later in flowering, a few failing entirely.

In the second generation there was extreme variation in the time of flowering of different individuals, some approximating the season of the early-flowering parent, and some failing to flower before frost. This great variability in the season of flowering made it difficult to classify the plants for characteristics that

change with the maturing of the individual. For this reason many plants were not noted at all. No records were kept of the exact flowering date of individuals in the second generation cultures.

Growth Habit

In most *oenothera* crosses, the individuals in the first and second generations fall into a comparatively few definite classes with respect to growth habit. Such was not the case in the crosses here described.

The first generation of the cross *grandiflora* $\times$ *franciscana* was remarkably uniform in every respect. In growth habit it was denser than *Oe. franciscana*, but definitely intermediate. The reciprocal cross, *franciscana* $\times$ *grandiflora*, differed chiefly in the development of green pigmentation. The plants were smaller and more open than in the former F_1 , but otherwise resembled it closely. The plants with relatively dark green leaves were indistinguishable from plants of the reciprocal cross.

Second generations were grown from each of the F_1 cultures. These, including double reciprocal F_2 cultures, totaled more than three hundred plants,¹ but it would have been practically impossible to find any two plants of strikingly similar growth habit. All intermediate types between the parental forms were present as well as many that were more extreme than either parent, the dwarfed plants being especially noticeable. No method was devised for recording the different habits of growth.

Bud Color

Oe. franciscana has brilliant red buds (Text Fig. 5 a); *Oe. grandiflora* has buds entirely green except for slight red markings on

¹ Most of the F_2 population came from the cross *grandiflora* $\times$ *franciscana*; culture 30, grown in 1924, had 77 individuals, and culture 262, grown the following year, had 136 individuals. Only 7 plants of the second generation of the reciprocal cross lived to flower; these belonged to culture 263, grown in 1925. Culture 37, with 36 individuals recorded, was the double reciprocal cross (*grandiflora* $\times$ *franciscana*) $\times$ (*franciscana* $\times$ *grandiflora*), a chlorotic plant being used as pollen parent. Culture 38, with 54 individuals recorded, was the same double reciprocal cross except that a dark green plant was used as pollen parent.

the most brightly illuminated side, and these are often lacking (Text Fig. 5 e).

All first generation plants had red buds, but of lighter color than the red-budded parent. F₁ culture 169 had 6 plants with

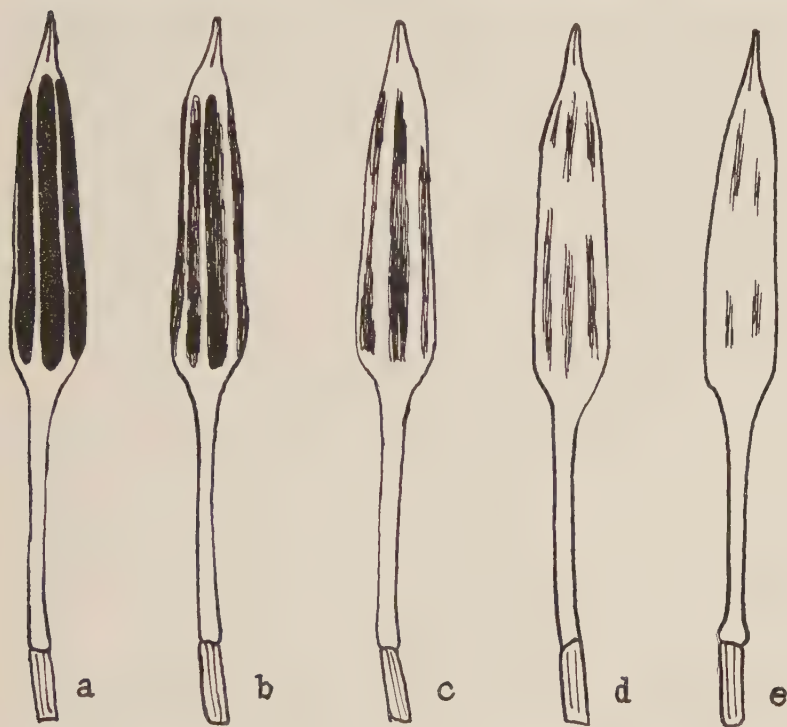


FIG. 5. Extent of red pigmentation on the buds of plants from the second generation of the cross *Oe. grandiflora* $\times$ *Oe. franciscana*. The red color is indicated by the black shading.

light red (b) buds and 3 with splashed (c) buds (compare Text Fig. 5).

Many of the plants in the second generation had buds intermediate in appearance between the red and green of the parents. In order to record the approximate color of the buds in the F₂ cultures, five "types" were selected with which the buds of any

plant might be compared. These types are illustrated in Figure 5 in the text. The F_2 frequencies for these five classes are given in Table I.

TABLE I
FREQUENCIES OF FIVE CLASSES OF BUDS IN THE SECOND AND
DOUBLE RECIPROCAL F_2 GENERATIONS OF THE CROSS
GRANDIFLORA $\times$ *FRANCISCANA*

Culture number 2	Red a	Light Red b	Splashed c	Light Splashed d	Green e
30	28	12	9	5	9
262	26	24	8	8	12
F_2 subtotal	(54)	(36)	(17)	(13)	(21)
37	11	11	5	7	2
38	16	15	9	7	7
double recip. subtotal	(27)	(26)	(14)	(14)	(9)
Total	81	62	31	27	30

Five F_2 plants of culture 30 were self-pollinated. F_3 progenies from F_2 plants with different bud colors gave varying frequencies, as shown in Table II.

TABLE II
FREQUENCIES OF FIVE CLASSES OF BUDS IN THE THIRD
GENERATIONS FROM F_2 PLANTS WITH DIFFERENT BUD
COLORS

Culture number	Bud color of parent	Red buds a	Light Red b	Splashed c	Light Splashed d	Green e
171	a red	15	12			
172	a red	20	4			
174	b lt. red	2				
170	e green (?)	3	5	13		9
173	e green	1	1	4		5

A back-cross, (*grandiflora* $\times$ *franciscana*) $\times$ *franciscana*, consisting of 14 plants, had 6 plants with red (a) buds, 2 with light red (b), 4 with splashed (c) and 1 with light splashed (d).

Sepal Tip Length

Oe. grandiflora has long sepal tips while those of *Oe. franciscana* are relatively very short (Pl. XXXV, Figs. 2 and 1). The

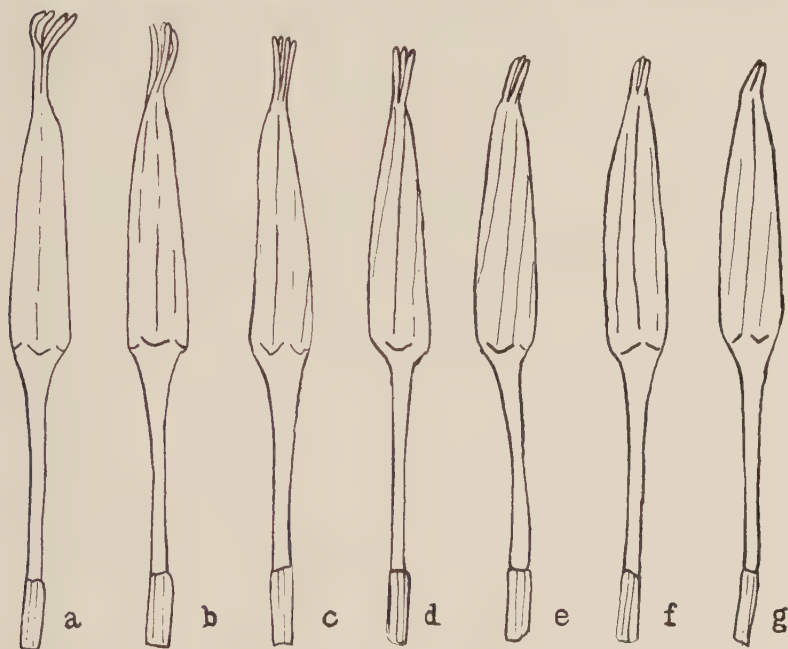


FIG. 6. Length of sepal tips on buds of second generation plants of the cross *Oe. grandiflora* $\times$ *Oe. franciscana*

first generation plants had buds of an intermediate nature, but with sepal tips much longer than the mean length of the F_2 population (Pl. XXXV, Fig. 3, and Text Fig. 6 b). F_1 culture 169 had 5 plants with rather long (b) sepal tips and 4 with very long (a) tips. In the F_2 there was great variation in the expression of this character. It proved difficult to make measurements with accuracy as there is no definite point at which the "tip" arises, and

there were great differences in total bud length. The general appearance of the tip of the bud seemed a better criterion for classifying the various types. Buds with short sepal tips are more sharply shouldered near the tip, while those with longer sepal tips are more tapering and have a stronger tendency to twist. There is also a difference in the firmness of the sepal tips, the short ones being tightly appressed and the longer ones more spreading (compare Pl. XXXV and Text Fig. 6). Seven arbitrary classes were chosen for convenience in classification. These are illustrated in Figure 6 in the text. The frequencies for the seven classes are given in Table III for the F_2 generations and in Table IV for the F_3 .

TABLE III

FREQUENCIES OF SEVEN CLASSES OF SEPAL TIPS IN THE SECOND, AND DOUBLE RECIPROCAL F_2 , GENERATIONS OF THE CROSS *GRANDIFLORA* $\times$ *FRANCISCANA*

Culture number	a (long)	b	c	d	e	f	g (short)
30	5	13	13	17	10	3	2
262	3	16	32	45	22	5	
F_2 subtotal	(8)	(29)	(45)	(62)	(32)	(8)	(2)
37	3	6	13	7	5	2	
38	3	12	14	9	11	5	
double reciprocal subtotal	(6)	(18)	(27)	(16)	(16)	(7)	
Total	14	47	72	78	48	15	2

TABLE IV

FREQUENCIES OF FOUR CLASSES OF SEPAL TIPS IN THE THIRD GENERATIONS FROM F_2 PLANTS WITH DIFFERENT LENGTHS OF SEPAL TIPS

Culture number	Sepal tips of parent	a	b	c	d
170	a	23	5	1	
171	b	19	8		
173	b	8	2		1
174	c				2
175	d	3	9	7	5

The back-cross (*grandiflora* $\times$ *franciscana*) $\times$ *franciscana* had 6 plants with *d* sepal tips, 4 with *e* sepal tips, 3 with *f* sepal tips, and 1 with *g* sepal tips.

Stem Tip Color

The young growing parts of the stem in the inflorescence of *Oe. franciscana* are marked with purple, the color disappearing on the older parts of the stem. The color is especially prominent in stripes just below the points of attachment of the youngest floral bracts, but in some cases the entire stem tip is uniformly colored. The coloring is more pronounced as the plant nears the end of its flowering season; late-flowering plants might be difficult to determine. *Oe. grandiflora* has green stem tips.

Plants of the first generation of crosses between these two species had stem tips intermediate in color between those of the parents. F_1 culture 169 had 2 plants classed as light purple and 6 as very light purple (compare with F_2 distribution).

The second generation did not segregate clearly into plants with green and purple stem tips, but many plants appeared intermediate. Some of the differences in color may have been due to differences in growth habit (the coloring is more noticeable on plants with a relatively open habit) and difference in season of flowering. Five arbitrary classes for degree of coloring were selected as an aid in classifying this character. These classes,

together with the frequencies in F_2 culture 262, were as follows: 4 "strong purple"; 26 "purple"; 17 "light purple"; 11 "very light purple"; and 17 "green"; totaling 75 plants recorded for this character.

Coloring of Stems at Base of Plant

The stems of *Oe. grandiflora* develop a diffused red color on all the older regions, especially near the base of the plant. This coloring varies in intensity with the exposure of the stems to sunlight and with the maturing of the plant. *Oe. franciscana* has green stems, often turning brown or slightly red late in the season. Plants of the first generations of crosses between these two species had the red stems like those of *Oe. grandiflora*. In culture 169, 7 plants were recorded with "red" stems and 2 with "light red" stems (compare with F_2 frequencies). Plants of the second generation gave every appearance of forming an intergrading series between red and green, though the extent of the variation due to density of foliage and season of flowering could not be determined.

Six classes were used in comparing the plants of the second generation for stem color. These classes with their frequencies in F_2 culture 262 were as follows: 6 "intense red"; 26 "red"; 16 "light red"; 6 "green with some red"; 28 "green with almost no red"; and 9 "green"; making a total of 91 plants recorded for this character.

Length of Floral Bracts

In the inflorescence of *Oe. franciscana* (Pl. XXXVI, Fig. 1) the young buds are shorter than the floral bracts subtending them. The buds elongate more rapidly than the bracts, until, on the day before opening, the buds are about one fourth longer than the subtending bracts. When the flower is open, the hypanthium is about equal to the bract in length. In *Oe. grandiflora* the buds are always longer than the floral bracts (Pl. XXXVI, Fig. 2). At first only the tips of the buds project beyond the bracts, but by the time of opening the bud is three or four times as long as the bract.

The first hybrid generation was intermediate for length of bracts (Pl. XXXVI, Fig. 3). Buds and bracts were of about equal length at first, and by the time of opening, the buds were about twice as long as the bracts.

Variation of the floral bracts in the second generation was nearly as great as the variation in growth habit (Pl. XXXVI, Figs. 3-5; Pl. XXXVII, Figs. 6-12). Some plants had bracts at first equaling or surpassing the buds, but which were not much greater than one fourth of the bud length at anthesis (Fig. 4). Some had bracts at first shorter than the buds, but growing longer than the hypanthia of the open flowers (Fig. 11). Some resembled *Oe. franciscana*, but were more extreme (Figs. 10 and 12), while others were more extreme in the opposite direction than the *Oe. grandiflora* parent (Fig. 6). The great majority of the F₂ plants were intermediate between these four extremes. In the field the floral bracts were classified in nine groups, based chiefly on the relative lengths of the youngest bracts and buds. These classes with the number of individuals in each in culture 262 were as follows: 4 "very long"; 26 "long"; 5 "rather long"; 23 "more than medium"; 8 "medium"; 5 "less than medium"; 6 "somewhat short"; 10 "short"; and 4 "very short"; making 91 individuals noted for this character.

Length of Buds

Oe. grandiflora and *Oe. franciscana* are fairly uniform for size of flowers and length of buds, but the second generations of crosses between the two were quite variable in both respects (Pls. XXXV-XXXVII). No measurements were made of flower size, but the bud cone of F₂ plants was measured, giving actually an indication of flower size. All measurements were made from herbarium specimens collected late in the summer of 1925, and only culture 262 was measured. The bud cone varied from 12 to 35 millimeters in length. Grouped in somewhat larger classes, the frequencies were as follows: 17 mm. or less, 21 plants; 18 to 20 mm., 20 plants; 21 to 23 mm., 29 plants; 24 to 26 mm., 30 plants; 27 to 29 mm., 18 plants; 30 to 32 mm., 2 plants; and 33 mm. or over, 3 plants; making a total of 123 plants measured.

Pubescence (Thick-walled)

The entire plant of *Oe. franciscana* is clothed with long thick-walled hairs. These are especially striking on the buds (Pl. XXXV, Fig. 1). *Oe. grandiflora* is almost entirely free from pubescence of this type. I failed to note carefully the pubescence of the plants of the first generation. Buds of F₂ culture 30 were examined under the microscope and the number of hairs per unit area were counted. The following is a summary of these counts: 1 hair per 10 units, 9 plants; 1 hair per 2 units, 2 plants; 1 hair per unit, 10 plants; 2 hairs per unit, 7 plants; 3 hairs per unit, 14 plants; 4 hairs per unit, 9 plants; 6 hairs per unit, 7 plants; and 8 hairs per unit, 5 plants; totaling 63 plants measured.

Pubescence (Thin-walled)

Both *Oe. franciscana* and *Oe. grandiflora* have another type of pubescence composed of numerous thin-walled secretory hairs. This type is also more numerous in *Oe. franciscana*, though the difference is not nearly as great as in the amount of thick-walled pubescence. The same buds used in the counts of thick-walled hairs were counted for thin-walled hairs, and the same unit of measure was used both times. Sixty-two plants were recorded for thin-walled pubescence; a summary of the counts follows: 4 hairs per unit area, 2 plants; 6 hairs per unit, 19 plants; 8 hairs per unit, 14 plants; 10 hairs per unit, 10 plants; 12 hairs per unit, 4 plants; 18 hairs per unit, 1 plant; 20 hairs per unit, 5 plants; and 25 hairs per unit, 7 plants.

EVIDENCE OF CORRELATION

Correlation between Five Characters

A group of five characters showed marked correlation in their inheritance. These were bud color, sepal tip length, stem tip color, basal stem coloring, and length of floral bracts. The characters coming into the cross from the *Oe. franciscana* parent were red buds, short sepal tips, purple stem tips, green stems, and long floral bracts. In all the following cases, the coefficients of correlation have been calculated in terms of the characters coming from this parent.

Red buds and short sepal tips: the coefficient of correlation, based on 63 individuals of F₂ culture 30, was $.314 \pm .076$.

The coefficient of correlation, based on 78 individuals of F_2 culture 262, was $.027 \pm .076$.

The coefficient of correlation, based on 36 individuals of the double reciprocal culture 37, was $.533 \pm .080$.

The coefficient of correlation, based on 54 individuals of the double reciprocal culture 38, was $.349 \pm .084$.

The coefficient of correlation, based on 231 individuals of all F_2 cultures, was $.244 \pm .042$.

Red buds and purple stem tips: the coefficient of correlation, based on 72 individuals of F_2 culture 262, was $.598 \pm .051$.

Short sepal tips and purple stem tips: the coefficient of correlation, based on 75 individuals of F_2 culture 262, was $.334 \pm .069$.

Red buds and green stems: the coefficient of correlation, based on 76 individuals of F_2 culture 262, was $.547 \pm .054$.

Short sepal tips and green stems: the coefficient of correlation, based on 89 individuals of F_2 culture 262, was $.377 \pm .054$.

Purple stem tips and green stems: the coefficient of correlation, based on 75 individuals of F_2 culture 262, was $.358 \pm .068$.

Red buds and long floral bracts: the coefficient of correlation, based on 75 individuals of F_2 culture 262, was $-.398 \pm .066$.

Short sepal tips and long floral bracts: the coefficient of correlation, based on 93 individuals of F_2 culture 262, was $-.071 \pm .069$.

Purple stem tips and long floral bracts: the coefficient of correlation, based on 72 individuals of F_2 culture 262, was $-.556 \pm .055$.

Green stems and long floral bracts: the coefficient of correlation, based on 87 individuals of F_2 culture 262, was $-.235 \pm .068$.

A summary of the relations of these five characters is given in Table V.

TABLE V
SUMMARY OF COEFFICIENTS OF CORRELATION FOR FIVE CHARACTERS

	Bud color	Sepal tip length	Stem tip color	Stem color
Length of floral bracts....	-.394	(-.071)	-.556	-.235
Stem color.....	.547	.377	.358	
Stem tip color.....	.598	.334		
Sepal tip length.....	.244			

Characters Independently Inherited

Red bud color and thick-walled pubescence: the coefficient of correlation, based on 62 individuals of F₂ culture 30, was $.044 \pm .085$.

Short sepal tips and thick-walled pubescence: the coefficient of correlation, based on 62 individuals of F₂ culture 30, was $.192 \pm .082$.

Red bud color and thin-walled pubescence: the coefficient of correlation, based on 61 individuals of F₂ culture 30, was $-.163 \pm .083$.

Short sepal tips and thin-walled pubescence: the coefficient of correlation, based on 61 individuals of F₂ culture 30, was $.252 \pm .079$.

Thick-walled and thin-walled pubescence: the coefficient of correlation, based on 62 individuals of F₂ culture 30, was $.356 \pm .075$.

Red bud color and long buds: the coefficient of correlation, based on 78 individuals of F₂ culture 262, was $-.095 \pm .075$.

Short sepal tips and long buds: the coefficient of correlation, based on 123 individuals of F₂ culture 262, was $.003 \pm .061$.

Purple stem tips and long buds: the coefficient of correlation, based on 75 individuals of F₂ culture 262, was $-.099 \pm .077$.

Green stems and long buds: the coefficient of correlation, based on 89 individuals of F₂ culture 262, was $-.217 \pm .068$.

Long floral bracts and long buds: the coefficient of correlation, based on 91 individuals of F₂ culture 262, was $.127 \pm .071$.

DISCUSSION

Multiple-Factor Interpretation

For the crosses reviewed in this paper, a multiple-factor interpretation is suggested in two distinct ways. In the first place, the F₂ distributions for certain characters resemble typical examples of multiple-factor inheritance. In the second place, when the inheritance of all the characteristics studied is considered collectively, it is evident that a great many factors must be involved.

The most striking multiple-factor distribution for the second

generation of these crosses is noticed in the inheritance of growth habit; but for this character the inheritance was only described, with no attempt at analysis. An almost parallel case appears in the inheritance of size of floral bracts, and here we know a little more about the inheritance. Both parents seem to contribute factors tending to increase the size of floral bracts, as shown by the segregates with extremely large bracts. *Oe. franciscana* has larger bracts than *Oe. grandiflora*, but largeness of bracts seems to be associated with other characters coming from *Oe. grandiflora* (Table V). The plausible explanation is that *Oe. grandiflora* carried dominant factors for small bract size (shown by the intermediate F_1) and also factors for large bract size, the latter associated in their inheritance with factors for green buds, green stem tips, and red stems. It is at least evident that the inheritance in this cross is not simple, and not due to one factor pair.

The inheritance of time of flowering, to judge from the F_2 distribution, would also seem to involve multiple factors. Here, again, the exact times were not recorded, but it was noted that both red- and green-budded plants, differing in sepal tip length, were among the first to flower. This is an indication of independence in inheritance between flowering season and the group of correlated characters shown in Table V.

The second generation, classified for bud color, gives a curve with the mode at the red end of the series. This is very different from the normal curve for multiple-factor inheritance, and it might be believed that a single factor pair was concerned in this cross if it were not for the frequencies in the back-cross to the red-budded parent (see p. 126). The presence of a large number of plants with intermediate bud colors in this back-cross suggests a difference of more than one factor pair between red and green bud colors.

The inheritance of bud cone length must also be due to several different factors. Both parents are somewhat similar for bud size, but it was noted that the second generation showed great variability for this character. It must be supposed that both parents contributed factors for both long and short buds.

Only two of the characters studied gave in F_2 definitely bimodal curves suggestive of single-factor differences with domi-

nance. These were stem color and thin-walled pubescence. In the former case there is a mode in the red region and another in the green, with approximately three fourths of the individuals having stems distinctly showing red. The case for thin-walled pubescence is more striking because of the break in the curve between the two modes. The frequencies of the classes having 4 to 12 hairs per unit area form a smooth curve. No individuals occur in the region between 12 and 18 hairs per unit. After this break, there is another group of individuals with its mean slightly above 20 hairs per unit area. The frequencies for these two groups are 49 and 13, respectively, a difference of but 2.5 from the theoretical 3 : 1.

The coefficients of correlation for the various character combinations may indicate very little when considered singly because of the uneven range of the arbitrarily chosen classes, but collectively they must be of some significance. It has been noted earlier that a group of five characters were correlated in their inheritance. With one exception (length of sepal tips and floral bracts), noticeable correlation is shown between every pair of the characters. These characters must either have certain factors in common or have factors that are closely associated in their inheritance.

All other characters studied seem to be independent in their inheritance, except that there seem to be correlation between the two types of pubescence and a possible indication of correlation between thin-walled pubescence and short sepal tips.

Since most of the characters studied seem to be the expression of several factors, and since many of the characters are independent in their inheritance and the others never completely associated, it is evident that a large number of factors have been segregating in the crosses described above.

Relation of Multiple Factors to the Paired Condition of Chromosomes

In a cross between two species differing in as many respects as the two discussed in this paper, it might well be expected that segregation for a great many factors would be shown in the second

generation. Such, however, is not ordinarily the case in the oenotheras. A cross between two oenothera species usually gives in F_2 comparatively few types of plants, each type differing from the others in a great many characteristics.

Unfortunately, the chromosome configuration in most crosses of species is not known. For *Oe. biennis*, *Oe. Lamarckiana*, and *Oe. muricata* (Auct. non L.), however, we do know the configuration of chromosomes, and in each of these there is little or no pairing of chromosomes at the reduction division. *Oe. Lamarckiana* has but one set of normally pairing chromosomes; the other two species have no pairing chromosomes (Cleland, 1923, 1925). Crosses between these three species show a large number of characteristics being inherited in blocs, the type of inheritance that is considered typical for the oenotheras. The crosses *Oe. muricata* $\times$ *Oe. Lamarckiana*, and *Oe. biennis* $\times$ *Oe. Lamarckiana* give in the first generation the twin hybrids *laeta* and *velutina*, both of which breed true when self-pollinated (De Vries, 1907; 1913, p. 126). Reciprocal crosses between *Oe. biennis* and *Oe. muricata* give first generations showing patroclinic inheritance for all characteristics except sepal tip length, which is matroclinic (Davis, 1914). None of the characters segregate in the following generation. The inheritance of sepal tip length is especially noteworthy, as there is no intergrading between the parental forms as seen in crosses between *Oe. franciscana* and *Oe. grandiflora*.

The crosses between *Oe. franciscana* and *Oe. grandiflora* appear to be the first between species which have been proved to have pairing chromosomes. Since this cross shows segregation for a great many factors, multiple-factor inheritance in the oenotheras may be concluded to be a function of pairing chromosomes.

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EXPLANATION OF PLATES

PLATE XXXV

Photograph of buds taken from herbarium specimens

- FIG. 1. Buds of *Oe. franciscana*
- FIG. 2. Bud of *Oe. grandiflora*
- FIG. 3. Buds of *Oe. franciscana* $\times$ *grandiflora* F₁
- FIG. 4. Buds of *Oe. grandiflora* $\times$ *franciscana* F₂
- FIG. 5. Bud of F₂ plant with semi-cruciate flowers

PLATES XXXVI-XXXVII

Tracings of buds and floral bracts made from herbarium specimens and reduced one half

- FIG. 1. *Oe. franciscana*
- FIG. 2. *Oe. grandiflora*
- FIG. 3. *Oe. franciscana* $\times$ *grandiflora* F₁
- FIGS. 4-12. *Oe. grandiflora* $\times$ *franciscana* F₂

PLATE XXXV



PLATE XXXVI



PLATE XXXVII



